

Application No. 10/724,301
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1-5, 9, 11, 16, 24 & 32 all.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

- ① (currently amended) A modified neomycin phosphotransferase gene wherein the modified neomycin phosphotransferase gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2 with the modification that the at amino acid encoded at position 91 and/or 198 and/or 240 in relation to the wild-type gene encodes for is a different amino acid than that of SEQ ID NO:2 the wild-type neomycin phosphotransferase gene.
- ② (currently amended) The modified neomycin phosphotransferase gene according to claim 1, wherein the encoded polypeptide modified neomycin phosphotransferase which is encoded by the neomycin phosphotransferase gene has a lower enzyme activity than the polypeptide with the amino acid sequence of SEQ ID NO:2 wild-type neomycin phosphotransferase.
- ③ (currently amended) The modified neomycin phosphotransferase gene according to claim 1 wherein the modified neomycin phosphotransferase gene ~~compared with the wild-type gene encodes:~~ encodes an alanine at amino acid position 91 and/or glycine at amino acid position 198 and/or isoleucine at amino acid position 240 in relation to the wild-type gene.
- ④ (original) The modified neomycin phosphotransferase gene according to claim 1 wherein the modified neomycin phosphotransferase gene encodes a polypeptide comprising an amino acid sequence according to SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:18.
- ⑤ (original) The modified neomycin phosphotransferase gene according to claim 1 comprising a sequence according to SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:17.

6. (withdrawn) A modified neomycin phosphotransferase gene wherein the modified neomycin phosphotransferase gene compared with the wild-type gene encodes: glycine or aspartic acid at amino acid position 182 and/or alanine or valine or glycine at amino acid position 227 and/or glycine or asparagine at amino acid position 261 in relation to the wild-type gene.
7. (withdrawn) The modified neomycin phosphotransferase gene according to claim 6 wherein the modified neomycin phosphotransferase gene encodes a polypeptide comprising an amino acid sequence according to SEQ ID NO:4, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.
8. (withdrawn) The modified neomycin phosphotransferase gene according to claim 7 comprising a sequence according to SEQ ID NO:3, SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:13.
9. (original) A modified neomycin phosphotransferase encoded by a modified neomycin phosphotransferase gene according to claim 1.
10. (withdrawn) A modified neomycin phosphotransferase encoded by a modified neomycin phosphotransferase gene according to claim 6.
11. (original) A eukaryotic expression vector containing a modified neomycin phosphotransferase gene according to claim 1.
12. (withdrawn) A eukaryotic expression vector containing a modified neomycin phosphotransferase gene according to claim 6.
13. (withdrawn) A eukaryotic expression vector containing a heterologous gene of interest functionally linked to a heterologous promoter and a modified neomycin phosphotransferase gene which codes for a neomycin phosphotransferase having a lower enzyme activity compared with wild-type neomycin phosphotransferase.

a)14. (withdrawn) A eukaryotic expression vector containing a heterologous gene of interest functionally linked to a heterologous promoter and a modified neomycin phosphotransferase gene which codes for a neomycin phosphotransferase having a lower enzyme activity compared with wild-type neomycin phosphotransferase wherein:

- (i) the modified neomycin phosphotransferase gene is a gene according to claim 1, or
- (ii) the modified neomycin phosphotransferase gene at amino acid position 182 and/or 227 codes for a different amino acid than the wild-type gene at the corresponding site, or
- (iii) the modified neomycin phosphotransferase gene at amino acid position 261 codes for a glycine.

13.15. (withdrawn) The expression vector according to claim 14, wherein by comparison with the wild-type gene the modified neomycin phosphotransferase gene at amino acid position 182 codes for glycine or aspartic acid and/or by comparison with the wild-type gene the modified neomycin phosphotransferase gene at amino acid position 227 codes for an alanine, glycine or valine.

16. (currently amended) The expression vector according to claim 11 ~~[[14]]~~, wherein the modified neomycin phosphotransferase gene encodes a protein comprising an amino acid sequence according to ~~SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:18, SEQ ID:20 or SEQ ID NO:22.~~

17. (withdrawn) The expression vector according to claim 14, comprising one or more enhancers functionally linked to the promoter.

18. (withdrawn) The expression vector according to claim 17 wherein the enhancer is a CMV or SV40 enhancer.

19. (withdrawn) The expression vector according to claim 13 wherein the promoter is a hamster ubiquitin/S27a promoter.

20. (withdrawn) The expression vector according to claim 19 wherein the heterologous gene of interest is under the control of the ubiquitin/S27a promoter.
21. (withdrawn) The expression vector according to claim 13 further comprising a gene for a fluorescent protein, wherein the gene for the fluorescent protein is, optionally, functionally linked to the gene of interest and the heterologous promoter.
22. (withdrawn) The expression vector according to claim 21, further comprising an internal ribosome entry site (IRES), wherein bicistronic expression of the gene which codes for the fluorescent protein and of a gene which codes for a protein/product of interest is enabled.
23. (withdrawn) The expression vector according to claim 21, wherein the gene which encodes the fluorescent protein and the gene which encodes the modified neomycin-phosphotransferase gene are located in one or in two separate transcription units.
24. (currently amended) A mammalian cell in vitro containing a modified neomycin phosphotransferase gene according to claim 1.
25. (withdrawn) A mammalian cell containing a modified neomycin phosphotransferase gene according to claim 6.
26. (withdrawn) A mammalian cell which has been transfected with an expression vector according to claim 14.
27. (withdrawn) A mammalian cell which has been transfected with an expression vector according to claim 21.
28. (withdrawn) The mammalian cell according to claim 27, further transfected with a gene for an amplifiable selectable marker.

29. (withdrawn) The mammalian cell according to claim 28, wherein the amplifiable selectable marker gene is dihydrofolate-reductase (DHFR).

30. (withdrawn) The mammalian cell according to claim 26, wherein the mammalian cell is a rodent cell.

31. (withdrawn) The mammalian cell according to claim 30, wherein the rodent cell is a CHO or BHK cell.

32. (currently amended) A method of enriching for a-mammalian cells that express a modified neomycin-phosphotransferase gene, comprising:

- (i) transfecting a pool of mammalian cells with a gene for a modified neomycin-phosphotransferase according to claim 1;
- (ii) cultivating the mammalian cells under conditions which allow expression of the modified neomycin-phosphotransferase gene; and
- (iii) cultivating the mammalian cells in the presence of at least one selecting agent which acts selectively on the growth of mammalian cells, and gives preference to the growth of the cells which express the modified neomycin-phosphotransferase gene.

33. (withdrawn) A method of enriching a mammalian cell, comprising:

- (i) transfecting a pool of mammalian cells with a gene for a modified neomycin-phosphotransferase according to claim 6;
- (ii) cultivating the mammalian cells under conditions which allow expression of the modified neomycin-phosphotransferase gene; and
- (iii) cultivating the mammalian cells in the presence of at least one selecting agent which acts selectively on the growth of mammalian cells, and gives preference to the growth of the cells which express the modified neomycin-phosphotransferase gene.

34. (withdrawn) A method of obtaining and selecting a mammalian cell which expresses at least one heterologous gene of interest, comprising: